CALCIUM AND PHOSPHORUS STUDIES WITH LACTATING DAIRY COWS

By

BARRY JEROME STEEVENS

Bachelor of Science

Wisconsin State University

River Falls, Wisconsin

1966

Submitted to the faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the degree of
MASTER OF SCIENCE
July, 1968

CALCIUM AND PHOSPHORUS STUDIES WITH

LACTATING DAIRY COWS

Thesis Approved:

L. J. Bush
Thesis Adviser

S. D. Musgrave

N. N. Durham
Dean of the Graduate College

ACKNOWLEDGMENTS

The author wishes to express sincere appreciation to Dr. Linville J. Bush for his competent advice, guidance, personal interest and invaluable assistance during the time of this study and the preparation of this thesis.

The author wishes to thank Dr. S. D. Musgrave, Head, Department of Dairy Science, for his interest, counsel, and guidance in this study and in all previous work. The author is grateful to Mr. Jack D. Stout for his interest, suggestions and assistance in this experiment.

The author is indebted to Dr. E. I. Williams for his sincere interest and veterinary service; to Mr. Joe Robinson and Mr. Harley Miller for care of the experimental animals; and to Mr. John Cunningham and Mrs. John Sandiford for their assistance with the laboratory analyses in this study. The author wishes to thank Mrs. Beverly Stowell for her work in the typing and editing of this study. Special appreciation is expressed to the author's wife, Kay, for her sacrifice, assistance, and encouragement during the course of this study and preparation of this thesis. Much appreciation is expressed to the author's parents, Mr. and Mrs. Jerome Steevens and to Mr. and Mrs. Herbert Nuernberger for their encouragement during this study.

The author is also grateful to Farmland Industries, Inc., Kansas City, Missouri, for providing funds for partial support of this research project.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The trend today in production is toward higher producing dairy herds which require a high level of feed intake. Many herds today are producing more milk than they did 30 years ago, placing increased demands upon the cow. As the cow's production potential is developed to a maximum, she often cannot obtain all of the essential nutrients which are needed. Dairy rations may vary from feeding a high level of roughage to feeding high levels of concentrates. Many times an imbalance of minerals result. Alfalfa contains a 6:1 or greater ratio of calcium to phosphorus, whereas most grains are relatively higher in phosphorus than calcium.

A young animal needs large amounts of calcium and phosphorus for growth of the skeleton. A cow during lactation secretes large quantities of calcium and phosphorus from her body into milk every day. A pregnant cow transfers a large supply of calcium and phosphorus to the growing fetus during the last 2 or 3 months of pregnancy. When these elements are in short supply, physiological disturbances result. As an example, parturient paresis may develop in a cow at or near calving and is associated with a condition of hypocalcaemia. The dietary ratio of calcium to phosphorus for dairy cows has been implicated as an important factor affecting the incidence of parturient paresis, reproduction and blood mineral composition. This study was undertaken to look at the effect of different ratios and levels of calcium and phosphorus in relation to parturient paresis, reproduction, milk production and blood mineral composition.

CHAPTER II

REVIEW OF LITERATURE

Introduction

The quantity of calcium and phosphorus in individual feedstuffs varies considerably in relation to each type of feed and the locality in which it is grown. Hill (1961) stated that cultivated pasturelands frequently contain over 1% calcium and about 0.4% phosphorus in the dry matter, whereas, in low fertility soil, the pasturelands may contain only 0.1% calcium and slightly less than 0.1% phosphorus. The amount of calcium in legumes differs considerably from that in the grasses. Alfalfa contains approximately 1.55% calcium and 0.24% phosphorus. Concentrates such as milo, barley, oats and wheat bran contain a much higher percentage of phosphorus than calcium. Thus, as feeding conditions change, imbalances of calcium and phosphorus may occur and must be corrected to satisfy the needs of the animal. Hill (1961) stated that as the maturity of the herbage increases, the phosphorus content falls. Also, the stem of the plant is found to be much lower than the leaves in calcium and phosphorus content.

Calcium and Phosphorus Metabolism

Availability

The availability of calcium and phosphorus refers to the "true" digestibility of the elements. By analogy with the determination of the digestabilities of organic constituents in feedstuffs, the values for calcium and phosphorus are also referred to as digestibility values, but, since no digestion of salts or ions is possible, the term "availability" is often used. Availability is defined as the amount truly absorbed by the animal.

The form in which calcium and phosphorus are supplied and also the levels fed seem to affect the availability to some degree.

Hansard et al. (1957) found the availabilities of various sources of calcium for mature and young animals as follows: bone meal, 55 and 68%; calcium carbonate, 40 and 51%; dicalcium phosphate, 50 and 64%; defluorinated dicalcium phosphate, 40 and 55%; ground limestone, 37 and 45%; and alfalfa hay, 31 and 41%. The various sources of calcium were fed in amounts equal to that required for maintenance. Visek et al. (1953) increased the calcium intake of dairy cows from 28 or 29 gm. daily to 120 gm. daily by the addition of steamed bone meal, resulting in a change in the true availability of calcium of the diet from 17 to 2% in one animal and from 56 to 19% in another. Other studies have shown very little difference in true digestibility when the daily intake of calcium changes (Lengemann, 1965). Six month-old calves were fed 10.2 and 30.4 gm. of calcium/day. The true digestibilities were 35.3 and 33.0% for the low and high levels, respectively. The daily fecal endogenous calcium loss remained nearly the same for the two levels of calcium intake. This experiment would suggest that the endogenous calcium is a function of the serum calcium level rather than being contolled directly by the level of intake. Lueker and Lofgreen (1961) reported that for growing lambs dietary calcium:phosphorus ratios of 0.8:1, 2.8:1 and 6.0:1 had no effect on the amount of calcium or phosphorus absorbed. The amount of either calcium or phosphorus absorbed was directly related to the amount fed. It was noted that the excretion of metabolic fecal calcium was independent of the amount of either calcium or phosphorus fed or absorbed, since it remained nearly constant on all diets. However, metabolic fecal phosphorus excretion was affected by the amount of calcium and phosphorus absorbed. The amount of metabolic fecal phosphorus excreted increased as the amount of phosphorus absorbed increased and decreased with increasing calcium absorption. The smallest excretion of metabolic fecal phosphorus occurred with the ration containing a 6.0:1 calcium:phosphorus ratio; however, the ratio of calcium-phosphorus absorbed was 1:1.

It is generally accepted that the true absorption of calcium and phosphorus is high in calves and decreases as the animals become older (Hansard et al., 1957). From previous work cited, it appears that the true absorption of calcium remains fairly constant in young animals which are depositing large amounts of calcium and phosphorus on the skeleton. In older animals, the true absorption of calcium decreases considerably when the level of intake is increased. Hansard et al. (1957) concluded that in general the differences in calcium availability for cattle from the various sources were not great. The differences due to source were somewhat more apparent in mature than in young growing animals. It was noted that availability values for calcium phosphate, bone meal and dicalcium phosphate rated highest for both age groups. Ground limestone, defluorinated calcium phosphate and calcium carbonate were intermediate between bone meal and the hay sources.

Long et al. (1957) compared the availabilities of several sources of phosphorus. Levels of 0.07, 0.11, 0.15 and 0.19% phosphorus were fed to young Hereford steers. No differences were found in availability of steamed bone meal, Curacao Island phosphate and dicalcium phosphate, in terms of a change of plasma phosphorus, weight gain, or feed intake. Feed intake, weight gain and plasma phosphorus all increased with increasing amounts of supplemental phosphorus in the ration. Ammerman et al. (1957) fed two commercial dicalcium phosphates, two calcined defluorinated phosphates, steamed bone meal, soft phosphate with colloidal clay, and Curacao Island phosphate to yearling beef steers at levels of 1 gm./100 lb. and 1.7 gm./100 lb. daily in two different trials. All were of equal value in promoting phosphorus retention and blood values of yearling steers. However, in a feeding trial with lambs soft phosphate and calcined defluorinated phosphate were poorly utilized, and gamma calcium pyrophosphate was essentially unavailable. It was observed that for dicalcium phosphate, Curacao Island phosphate, soft phosphate and defluorinated phosphate the "true" absorption values were 100, 90, 50 and 54%, respectively. Vitreous calcium metaphosphate was utilized to some extent, but significantly less than monocalcium phosphate. Dutton et al. (1967) stated that the form of dietary phosphorus had no significant effect on the absorption and retention of magnesium and calcium and on the absorption of phosphorus. However, phosphorus retention was higher for rations containing inorganic phosphorus supplements than for rations with organic phosphorus sources. Arrington et al. (1962) fed three-month-old dairy calves dicalcium phosphate, Curacao Island phosphate and defluorinated phosphate and found the "true" absorption of phosphorus by means of a depletion technique, as described by Ammerman et al. (1957), to be 98.2, 85.2, and 82.7%, respectively. Tillman and Brethour (1958[a]) found the availability of phosphorus supplied by phosphoric acid to be in the same order of magnitude as that supplied by dicalcium phosphate. In yearling Herefords, the true digestibility for phosphoric acid was 76.3% and 75.2% for dicalcium phosphate. Wise et al. (1961) reported that less than one-half of the phosphorus found in soft phosphate with colloidal clay was available to Holstein calves.

There is some question as to the availability of the organic form of phosphorus called phytin phosphorus. Phytin phosphorus is poorly utilized by nonruminants, but is utilized to some extent by ruminants. Tillman and Brethour (1958 found that the true digestibility of phosphorus in calcium phytate by sheep was 63% as compared to 70% for monocalcium phosphate. The true digestibility of calcium in calcium phytate was 52% as compared to 49% for monocalcium phosphate. Raun et al. (1956) found the phosphorus in calcium phytate to be as available to rumen microorganisms as was inorganic phosphorus. The optimum pH for phytase activity was approximately 5.5. Tillman and Brethour (1958b) found that 92% of the phytin phosphorus was hydrolized in an "in vitro" study.

Hill (1961) stated that a number of experiments in which the availabilities of calcium and phosphorus were determined on the same diet confirm the general trend of a higher availability of phosphorus than calcium. Availability data for calcium and phosphorus may apply only to feeding conditions similar to those of the experiment and may bear little or no relation to the properties of the substances fed. Meta and pyrophosphates are lower in availability than ortho-phosphates because they must be converted to ortho-phosphates before they can be absorbed.

Balance Studies

Isotope studies have been useful in determining the true absorption and retention of calcium and phosphorus. The method reported by Hansard et al. (1954) for estimating the excreted calcium and its partition within the gastrointestinal tract consists of integrating the excretion data from animals dosed orally with that of similar animals receiving a single intravenous dose of labeled calcium. Višek et al. (1953) determined engodenous fecal calcium in cattle by a simple isotope dilution method, wherein the animals were given daily injections of $Ca^{45}Cl_2$ intravenously. A similar method employing P^{32} was used by Kleiber et al. (1951) for determining the endogenous phosphorus excretion in cows. This experimental method assumes that the calcium or phosphorus excreted into the intestine, regardless of the pathway, has the same specific activity as the element in the plasma. This assumption is supported by the observation of Lueker and Lofgreen (1961) that the specific activity of the phosphorus of saliva was a good indication of the specific activity of the phosphorus of the blood serum 10 days after a subcutaneous injection of P^{32}.

Use of long-term balance studies is another approach that has been used to study the absorption and retention of calcium and phosphorus. Ellenberger et al. (1931) reported the results of long-term balance studies with dairy cows. One of the limitations of such studies is the cumulative error of the balance trials. Duncan (1958) noted that at the end of a three-year cumulative balance study on one particular cow there was some discrepancy between the balance results and final carcass analysis.

One important factor that must be kept in mind when evaluating the balance studies is the effect of age. Hansard et al. (1954) reported the true digestibilities of calcium for various age groups as follows: 30 days, 98%; 6-month, 41%; yearling, 34%; 2-years, 36%; mature, 34%; and aged, 22%. It was observed that as the animal advanced in age the endogenous loss increased and in the aged animals the endogenous loss exceeded the absorption from the tract, resulting in a negative balance. With Hereford cattle the per cent of the calcium intake excreted at 10 days, 6 months and at maturity was 7.2, 76 and 103%. The daily endogenous fecal calcium at 10 days, 6 months and maturity was 0.53 gm./100 lb., 0.84 gm./100 lb. and 0.80 gm./100 lb., respectively. Therefore, if the absorption decreases and the endogenous secretion increases in the mature animal, the amount of calcium retained is going to be much less. As noted earlier, the true digestibility of calcium by a calf was about 98% and that by a mature cow was about 34%. Part of this difference could be accounted for, as noted by Hill (1961), as a difference in the type of food given, namely milk to calves, and hay and grain to growing stock. Work was cited wherein replacing the soybean meal in a hay and concentrate ration by dried skimmilk increased the availability of calcium of the entire ration from 8 to 23% in an aged cow. In balance studies conducted by Visek et al. (1953), the true digestibility of calcium by yearling steers varied from 19 to 50% and in 8-year-old dairy cows the true digestibility was 2 to 56%.

The calcium and phosphorus balance of a lactating dairy cow is dependent upon the age, growth and milk production. Forbes and Beegle (1916) conducted a study in which they found that the calcium in the feces was nearly the same as the intake calcium. Heavy producing cows were in a negative balance even though sufficient calcium was supplied by the ration. Huffman et al. (1930) studied the calcium and phosphorus metabolism of cows producing 25 to 35 kg. of milk daily. The per cent of calcium retained was about 34.2 to 57.6%. On the basis of some previous balance studies, it was concluded that the actual intake has a much greater significance than the calcium:phosphorus ratio. The correlation of per cent calcium and per cent phosphorus retained in relation to intake was 0.90 and 0.81, respectively. The correlation for per cent calcium and per cent phosphorus utilized in relation to the calcium-phosphorus ratio was 0.57 and 0.39, respectively.

In some of the early balance studies by Ellenberger et al. (1931) a lactating dairy cow was in a negative balance in early lactation and in a positive balance later in lactation. By supplementing the ration with steamed bone meal or ground limestone the negative balance period was shortened. It is often assumed that because the ratio of calcium-phosphorus in bone is of the order of 2:1, intake, retention and loss should be in the same ratio, but there appear to be mechanisms which may allow storage of an excess of either element, and in bone some 15% of the calcium is not bound to phosphate (Duncan, 1958). Ellenberger et al. (1950) reported that the skeleton contained about 84% of all the phosphorus in the body and about 98% of the total calcium. The calcium-phosphorus ratio of the body was about 1.6:1 in a calf and 1.9:1 for a mature animal. Duckworth and Hill (1953) concluded that when minerals were lost from bone both the matrix and the mineral matter were removed simultaneously. This led to the general idea that according to the mechanism by which mineral matter is lost from the skeleton, bone weight and ash weight should be reduced, but not ash percentage. Eckles et al.

(1926) found a high calcium:phosphorus ratio in the bones of dairy cows fed on a low phosphorus diet. Meigs et al. (1935) reported a small change in the calcium:phosphorus ratio during resorption. The change in cattle was from 2.15:1 to 2.05:1. Duckworth and Hill (1953) concluded that a decrease in the calcium:phosphorus ratio during resorption is more common than an increase. Manston (1967) reported that the absorption of calcium or phosphorus by dry nonpregnant cows increased when the dietary intake of the element increased, but only for a few days. In long-term experiments with pregnant heifers feeding dietary calcium:phosphorus ratios of 2:1 and 1:1, better absorption of both elements was obtained from the 2:1 ratio diet. This agrees with other studies (Dowe et al., 1957; Wise et al., 1963) which have shown a decrease in performance and nutrient utilization by Hereford calves when calcium:phosphorus ratios were less than 1:1 or greater than 7:1. In a field trial by Manston (1967), a high calcium diet fed the last week of pregnancy was found to have no beneficial effects on the concentrations of plasma calcium or inorganic phosphorus at parturition.

Duncan (1958), in summarizing the work of Ellenberger et al. (1931), reported that there was no quantitative relation between the amount of calcium and of phosphorus apparently retained and certainly no sign of a 2:1 ratio between them. The calcium intake appeared to affect the amount of calcium retained. When mineral supplements were given, more calcium was always retained than when the animal was on a basal diet. On the other hand, neither calcium nor phosphorus intake levels seemed to influence the retention of phosphorus in this particular study. Some cows appeared to retain more phosphorus on the basal diet than when they were supplemented with bone meal and ground limestone. Duncan (1958) concluded that the mineral supplements had no significant effect on the mean daily milk yield. Westerlund (1937) concluded that the most notable result is that there is no constant optimum value for the calcium-phosphorus ratio. If the animal is to absorb the same amount of calcium at both high and low ingestion of calcium, a larger amount of total phosphorus is required. Therefore, with a low ingestion of calcium the resulting calcium-phosphorus ratio would be narrower than if a high level of calcium were ingested. From a statistical analysis of other work, Westerlund (1956) found that with more food phosphorus, the fecal phosphorus increased and when the calcium loss increased there was an increase in fecal phosphorus. Also, when more milk phophorus was secreted there was a decrease in fecal phosphorus and an increase in calcium loss. It was noted that the amount of phosphorus in milk was considered to be a reflection of the stage of lactation.

Vitamin D

The role of vitamin D in calcium assimilation has been recognized for some time. Meigs et al. (1926) noted that calcium assimilation is facilitated when there is present in the ration a vitamin which is contained in fresh green plant material. It is generally agreed that vitamin D enhances the intestinal absorption of calcium and directly influences calcium and phosphorus metabolism by the skeleton and kidney (Wasserman, 1962). Wasserman (1962) concluded that in a rat vitamin D does not act through an active transport system. Calcium was observed to pass from the plasma to the lumen and from the lumen to the plasma. The total driving force of the calcium ions was dependent upon the concentration gradient, electropotential gradient, solvent drag and hydrostatic pressure.

Manston (1964) studied the effects of vitamin D on calcium and phosphorus in two dry nonpregnant Ayrshire cows. The cows were fed 50 gm. of calcium and 16 gm. of phosphorus per day. The per cent of intake absorbed for calcium and phosphorus was 17.6 and 32.5%. The addition of vitamin D to the ration increased the absorption of calcium and phosphorus to 25.5 and 65.5%, respectively. The plasma calcium increased from 9 mg./100 ml. to 11 mg./100 ml. and then came back down to 9 mg./100 ml. again. The rate of bone accretion and resorption increased from 0.68 to 1.0 gm./hr. When vitamin D was fed there was no reduction in endogenous calcium secretion by the animal. Hibbs and Conrad (1960) studied the effects of feeding massive doses of vitamin D to cows for a period of seven days in an effort to reduce the incidence of milk fever. They fed three levels of 15, 20, and 30 million International Units (I.U.) per day. The protection against milk fever was accomplished by increasing the absorption and retention of calcium for a short time, thus building up the labile calcium reserve of the animal. Hibbs and Conrad (1966) observed that with a positive phosphorus balance the addition of vitamin D increased the absorption of phosphorus and calcium, but in animals with a negative phosphorus balance the addition of vitamin D actually decreased the absorption of both calcium and phosphorus.

There has been some concern over the safety of feeding massive doses of vitamin D. Excessive amounts of vitamin D increase absorption, build up the labile calcium reserve, but also result in calcification of the soft tissues. Payne and Manston (1967) fed low producing cows rations containing 60 gm. of calcium, 16 gm. of phosphorus and 13 gm. of magnesium/day. When a 40 million I.U. dose of vitamin D was fed the results were fatal. A single dosage of 10 million I.U. resulted in some lesions and metastatic calcification, but up to three doses of 10 million I.U. were fed without any visible clinical effects. When a different level of minerals was fed, i.e., 40 gm. of calcium, 30 gm. of phosphorus and 16 gm. of magnesium/day, a 10 million I.U. dose produced no lesions, whereas a 20 million I.U. dose produced some lesions. When the level of phosphorus was lowered from 30 gm. to 18 gm./day a 10 million I.U. dose again resulted in some lesions. It appears from this study that when the level of phosphorus is low in the ration there is a greater incidence of calcification of soft tissues.

Hill (1961) summarized several studies in which sheep were fed vitamin D and concluded that the dietary requirement for calcium was less in the presence of ample vitamin D than when the vitamin was in short supply.

Parathyroid Function

Several experiments indicate that the parathyroid hormone partially controls or regulates calcium and phosphorus in the body (Stott and Smith, 1957b; Perry et al., 1966). Stott and Smith (1964) stated that there are cyclic changes that occur in the histological structure of the parathyroid glands which correspond with the annual mineral metabolism of the cow. During heavy lactation the appearance of the glands was indicative of depressed secretory activity. After mid-lactation the size of the glands increased and just prior to parturtion the parathyroids showed evidence of the greatest secretory activity.

Stott and Smith (1957a) observed that parathyroidectomy of lactating cows resulted in a marked decrease in serum calcium, but the serum calcium came back up to normal shortly thereafter. Parathyroidectomy appeared to have little influence on milk production. This result led to the question of whether or not all of the parathyroid gland tissue was removed. Stott and Smith (1957[b]) performed a partial parathyroidectomy of dairy calves between 8 and 90 days of age, and observed no tetany and little effect on the serum calcium and phosphorus. A complete thyro-parathyroidectomy on dairy calves resulted in a marked lowering of the serum calcium and a smaller decrease of serum phosphorus. Tetany and death followed shortly, but it was noted that the serum phosphorus rose just prior to death. Before death the calcium and phosphorus levels were 6.4 and 8.0 mg./100 ml. At death the serum calcium and phosphorus levels changed to 6.0 and 12.0 mg./100 ml., respectively. Pischke and Stott (1964) removed the parathyroid and the thyroid glands from two lactating cows. Thyroactive casein was fed at the rate of 10 gm./day to compensate for the removal of the thyroid. No difference in milk calcium or phosphorus was effected by increasing the calcium or phosphorus intake or by varying the serum calcium and phosphorus by use of a chelating agent. When dairy cows were infused with disodium ethylenediamine tetra-acetate (EDTA), an agent used to tie up calcium and decrease the serum calcium level, the serum calcium values did not fall as rapidly nor return to the prechelation levels as rapidly in the parathyroidectomized cows as in the normal cows (Brown and Smith, 1964). Injection of $Ca^{45}Cl_2$ in parathyroidectomized cows resulted in a slower excretion of Ca^{45} than with normal cows. Mayer et al. (1967) observed that the administration of a parathyroid extract consistently produced an elevation of plasma calcium in both intact and parothyroidectomized cows. Following cessation of extract administration a decrease in plasma calcium to below control levels was observed. This was most pronounced in the parathyroidectomized cows where the plasma calcium fell to as low as 1.75 mmoles /1. It was concluded that the increased fecal calcium during parathyroid extract administration was the result of both decreased absorption and increased endogenous secretion, whereas the more elevated fecal calcium output following parathyroid extract treatment appeared to be solely the result of diminished calcium absorption. It appears from this study that there may be other regulatory mechanisms which were responsible for bringing the blood mineral levels back up to normal again in the parathyroidectomized cow.

Another hormone, calcitonin, is produced by the parathyroid gland (Pearse, 1966). Calcitonin is believed to act as a fine regulator of the calcium homeostasis. Care et al. (1967) reported that hypercalcaemic perfusion of an intect thyroid gland in a pig resulted in a hypocalcaemic response of the systemic system. This response reached a maximum in about two hours. They concluded that the secretion of calcitonin acts as a negative feedback of calcium. An injection of a calcitonin extract resulted in decreased blood calcium and blood phosphorus levels.

Requirements

Calcium and Phosphorus Requirements for Maintenance

Estimates of the requirements of calcium and phosphorus have been made using a variety of methods, and numerous criteria have been used. Hill (1961) suggested that in evaluating the requirements one must consider age, lactation, reproduction, maintenance and availability of calcium and phosphorus. Tillman et al. (1959) emphasized the desirability of expressing requirements of calcium and phosphorus as percentages of dry matter rather than in absolute amounts. Further precision could be obtained by relating the values to available energy rather than dry matter.

The present recommended allowance for a 550 kg. mature cow for maintenance is 15 gm. of calcium/day based upon 1965 N.R.C. recommendations, which assumes 50% true absorption of calcium. In addition, if the cow is producing 20 to 35 kg. of milk daily, an allowance of 2.4 gm. of calcium per kg. of milk must be added. For the last 2 or 3 mo. of pregnancy 13 gm. of calcium is allowed. Therefore, if a 550 kg. cow in the period of heavy lactation is producing 25 kg. of milk/day, her total requirement for maintenance and milk production would be 75 gm. of calcium/day.

When balance experiments are used to establish levels of calcium and phosphorus for lactating dairy cows, the stage of lactation must be given careful consideration. Forbes and Beegle (1916), Huffman et al. (1930) and Ellenberger et al. (1931) all noted that the dairy cow producing in the peak of her lactation usually had a negative balance of calcium and phosphorus even if the amount present in the diet was sufficient to meet the requirements.

Huffman et al. (1930) found the per cent of retention of calcium to be about 34 to 58% when timothy hay was fed and 14 to 34% when alfalfa was fed. This difference is apparently a reflection of the total amount of calcium consumed. From this study, it was concluded that the per cent retained was much greater when there was a greater need, and when a larger amount is fed the per cent of the total intake retained is less. Moodie (1960) reported that in a lactating cow the serum contains about 1.5 to 2.0 gm. of calcium. The interstitial fluid contains about 6 to

10 gm., and the absorption from the alimentary canal amounts to about 15 to 30 gm./day. Therefore, if a cow is excreting 15 to 30 gm./day in milk and 8 to 12 gm. as endogenous fecal calcium, the cow is dependent upon the amount abosrbed and the labile bone calcium supply to fulfill the requirements. Bone contains about 2.1 gm. of calcium/1.0 gm. of phosphorus and these elements exist in this approximate ratio even in a severe and prolonged deficiency of calcium. However, balance studies indicate that these two elements are usually resorbed from the skeleton in a much wider ratio than 2:1 (Reid, 1962). This could be partially explained if the soft tissues of the body gain phosphorus as a result of bone demineralization or if $CaCo_3$ in the bone is resorbed first (Reid, 1962). The latter possibility could account for 300 to 500 gm. of calcium without the loss of any phosphorus from the bones (Meigs et al., 1935).

In an example of over-estimation of retention of the elements, Reid (1962) discussed one cow in a long-term calcium balance study by Ellenberger et al. (1931), where the storage of calcium was over-estimated by 3447 to 5147 gm. Considering the total intake, the measurement error is relatively small; however, calcium allowances based upon balance data may be too low.

Hansard et al. (1954) and Visek et al. (1953) indicated that the amount of fecal endogenous calcium per unit of body size increases progressively as an animal becomes older. In reviewing the above work relative to calcium requirements, Reid (1962) stated that between 6 mo. and maturity the average daily endogenous excretion was about 0.7 gm./ 100 lbs. of body weight and the true absorption averaged about 35%. Since the fecal metabolic calcium constitutes most of the calcium lost by the cow the minimum dietary requirement for maintenance was estimated to be on the order of about 2 gm./100 lbs./day. For aged cows the fecal calcium was about 1 gm./100 lbs of body weight and the true absorption approximately 22%. On this basis, the minimum requirement for aged cows would be 4.5 gm./100 lbs.

Manston (1967) fed four dry nonpregnant cows various levels of phosphorus and calcium ranging between 20 to 92 gm. of phosphorus and 47 to 124 gm. of calcium/day. It was observed that the percentage of dietary calcium absorbed (30%) remained fairly constant in each cow resulting in an increase in the total quantity of calcium absorbed as the dietary intake increased. The difference between calcium absorption as measured by calcium-45-excretion, and net calcium absorption, as measured by total fecal calcium, indicated that the endogenous loss of calcium into the feces increased slightly during the higher calcium intakes. The absorption of phosphorus (30 to 60%) was not affected by variations in calcium intake. When 20 gm. of phosphorus/day were fed to a dry nonpregnant cow, there was a negative phosphorus balance, but pregnant hiefers fed 20 gm. of phosphorus/day maintained quite adequate absorption of phosphorus. This difference was explained as a possibility of a difference in absorption due to pregnancy, a difference in availability from different concentrates, or a difference of absorption due to a difference of age.

Since samples of blood are very readily obtainable, numerous studies have been made wherein blood levels were the criterion of adequacy of different mineral elements. It is well recognized that plasma phosphorus reflects phosphorus intake more rapidly than plasma calcium reflects calcium intake, but in either case the values should be considered in relation to the physiological state of the animal (Hill, 1961). Duncan (1958) stated that bone mineral is readily mobilized to maintain the level of serum calcium, but less readily to maintain that of phosphorus so that a low serum inorganic phosphorus level is the first sign of a deficiency of phosphorus. Lane et al. (1968) observed that the trichloracetic acid soluble whole blood mineral composition of Guernsey cows for phosphorus, magnesium, calcium, sodium and potassium were 6.1, 2.3, 7.4, 281 and 52 mg./100 ml., respectively. A significant effect of month of pregnancy was found for phosphorus, sodium, and potassium. A significant effect for age was found for phosphorus and potassium, and for month of lactation for phosphorus, magnesium, calcium and sodium. Payne and Leech (1964) observed in Ayrshire and Friesian cows the blood mineral values for calcium and phosphorus were 10.65 mg./100 ml. and 5.23 mg./100 ml., respectively. They concluded that a difference in calcium and phosphorus exists between herds, stage of lactation and age. As the animal increases in age, there is a slight decrease in calcium and a reduction of phosphorus. Palmer et al. (1930) noted day to day variations of inorganic blood phosphorus from 4.2 to 5.2 mg./100 ml. Exercise first produced a rise in blood phosphorus and then a decline one hour later. It was observed with calves that the inorganic blood phosphorus was 5.1 mg./100 ml. at birth, 7.7 mg./100 ml. at three days of age, 7.3 mg./100 ml. at 10 weeks and then back down to 6.1 mg./100 ml. when they were six months or older (Malan and Green, 1928; Palmer et al., 1930). However, these variations may be a reflection of feed intake.

Heifers on a phosphorus deficient pasture had a blood phosphorus level of 2.3 mg./100 ml., whereas heifers fed bone meal had a normal level of 5.0 mg./100 ml. (Malan et al., 1928). The calves born of the deficient heifers were all right as long as they received milk from the cow.

Sheep fed a calcium deficient diet had a linear blood calcium decrease for three weeks and then returned to normal for the next seven weeks (Nelson and Tillman, 1967). They noted that the renal clearance for phosphorus was greater in calcium deficient sheep.

Requirements for Milk Production and Reproduction

Milk contains about 0.54 gm. of calcium and 0.46 gm. of phosphorus/ lb. It is generally agreed that the calcium and phosphorus of milk remains fairly constant and presumably a very severe shortage of calcium would be reflected in a decrease in milk volume (Hill, 1961). Becker et al. (1934) reported that the production of Jersey cows receiving about 14 gm. of calcium and 45 gm. of phosphorus/day and producing 20 to 25 lb. of milk/day was increased about 4 lb. by the addition of 2% bone meal to the ration. Hill (1961) questioned the validity of the conclusion drawn from this observation of Becker et al. (1934) because none of the cows remained as controls without bone meal after the 2% bone meal supplement was added. While the animals were on the deficient diet there was a high incidence of weak bones which were depleted of calcium and phosphorus.

It appears that a cow is able to adjust the per cent utilization to the amount of calcium available. For example, in a Wisconsin experiment, Hart (1932) reared dairy cattle to 6 to 8 mo. of age on a low calcium diet and fed a winter ration containing 25 to 28 gm. of calcium/ day during the productive life. The cows on the low calcium diet produced as much milk as those fed a high calcium ration of 95 to 100 gm. of calcium/day during lactation. Also, the skeletons of the low calcium group contained as much calcium and phosphorus as did the high calcium group. They explained this difference as an increase in efficiency of utilization of calcium by the low calcium group. Converse (1954) observed that when cows were fed a ration containing about 0.26% calcium in the dry matter of the feed, milk production was not impaired, and in one cow about 50% of the calcium intake was put into the milk. In another experiment feeding young cows 0.15 to 0.19% calcium on a dry matter basis had no adverse effect on milk production. One cow secreted 87% of the feed calcium into the milk. It was concluded that the minimum requirement of calcium for milk production on a dry matter basis was about 0.16% calcium. Lamb et al. (1934) noted that cows were able to utilize phosphorus more efficiently on a ration low in this element. Lactating cows receiving 21 gm. of phosphorus/day utilized 56.7% of the phosphorus whereas cows receiving 47 gm. of phosphorus/day utilized 41.2% of the element. Fitch et al. (1932) observed that in lactating cattle receiving rations containing 0.32% and 0.64% calcium on a dry matter basis, no significant difference in milk production or milk composition was observed. Comar et al. (1966) fed 121 gm. and 54 gm. of calcium to first lactation dairy cows producing 22.6 and 21.8 lb. of milk/day, respectively. About 58% of the milk calcium originated from the diet in the high calcium group whereas in the low calcium group 42% of the milk calcium came from the diet. This would suggest that a higher dietary calcium level would result in a reduced drain on the body calcium reserves for secretion into milk.

In view of the calcium and phosphorus requirements for reproduction, very low levels of calcium and phosphorus are needed before reproduction is impaired. Webster (1932) concluded that in heavy milking cows bovine sterility may be caused by a phosphorus deficiency. A level of 1% calcium and 1% phosphorus in the ration was recommended to reduce sterility. Fitch et al. (1932) reported that a ration containing approximately 0.18% calcium fed for a period of three years did not decrease the breeding efficiency of a group of seven cows. Converse (1954) concluded that the minimal level of calcium for gestation appeared to be 0.16% calcium on a dry matter basis in the total ration. Eckles et al. (1935) stated that a phosphorus deficiency in the cattle studied did not influence the estrus cycle. It was concluded that the disturbances in estrum and the low calf crop among cattle in phosphorus deficient areas under natural conditions are probably due to the complicated nutritive deficiencies prevalent under such conditions and not to a phosphorus deficiency alone. Huffman et al. (1933) observed that with a 0.20% level of phosphorus in the ration no reproductive disturbances due to a phosphorus deficiency were observed. Palmer et al. (1935) reported that three cows had reproduced normally on a ration of 0.18% calcium, but when the ration was lowered to 0.12% calcium there was evidence in the form of dead calves at birth that the 0.12% level was too low for successful reproduction.

Hignett and Hignett (1952) suggested that impaired fertility due to a high calcium:phosphorus ratio with a relatively low phosphorus level might be corrected with an adequate supply of vitamin D. Later Hignett (1959) suggested an imbalanced calcium:phosphorus ratio along with a low manganese level might cause a decrease in fertility. Littlejohn and Lewis (1960) pointed out that a high calcium:phosphorus ratio depressed the growth rate but did not have any effect on the conception rate. In one trial involving a wide calcium:phosphorus ratio, the total daily intake for a 1000 lb. animal was approximately 9 gm. of phosphorus and 106 gm. of calcium.

As to the amounts of calcium and phosphorus needed for pregnancy, large quantities of calcium and phosphorus are not stored in the fetus until near the seventh month of gestation. Symonds et al. (1966) reported that the calcium transfer rate to a near-term fetal calf as measured with radioisotopes was 5.3 gm./day and the phosphorus transfer rate measured in two other cows was 1.7 and 2.6 gm./day. In reviewing the calcium and phosphorus requirements for pregnancy, Reid (1962) assumed that the true absorption of calcium was 35% and the true absorption of phosphorus was 50%. On this basis, the daily dietary needs of the cow for the fetus are 12 gm. of calcium and 4.4 gm. of phosphorus at the seventh month of gestation. Near the end of gestation the requirement for the fetus increases to 40.6 gm. of calcium and 13.4 gm. of phosphorus. The 1965 N.R.C. recommendations for the growing fetus in the last 2 or 3 mo. of pregnancy for a 550 kg. cow are about 13 gm. of calcium and 11 gm. of phosphorus/day.

Parturient Paresis

Parturient paresis is essentially a condition of hypocalcaemia in which the calving cow fails to obtain sufficient calcium from the gut or bone (Moodie, 1960). Since the blood serum contains only 1.5 to 2.0 gm. of calcium and the interstitial fluids contain only 6 to 8 gm. of calcium the cow is dependent upon the remainder from either resorption of labile bone calcium or absorption from the alimentary canal (Moodie, 1960).

Theories as to the cause of milk fever include parathyroid deficiency, liver dysfunction, and a condition of bowel stasis in which the digestive tract is unable to reabsorb sufficient amounts of calcium (Moodie, 1965). Moodie (1960) noted that the degree of hypocalcaemia was more crucial to development of paresis than was the duration. Cows known to have a previous history of paresis were held below 8 mg./100 ml. of calcium for some time, but when the serum calcium dropped below 5 mg./ 100 ml. paralysis occurred shortly. The conditions of an animal with milk fever are hypocalcaemia, hypophosphataemia, hypoproteinaemia, hyperglycaemia, a low concentration of citric acid, a high concentration of pyruvic and lactic acid and also hypomagnesaemia (Moodie, 1960). Constipation is a well known feature of milk fever and is generally assumed to be the result of the hypocalcaemia, since the injection of calcium is frequently followed by evacuation of the bowels. However, this does not exclude the possibility that stasis of gut movement at the time of calving impairs absorption of calcium and leads to a condition of hypocalcaemia.

Blood levels of calcium, phosphorus, potassium and sodium in parturient paretic cows at parturition are 7.76, 2.77, 23.7 and 323 mg./100 ml., respectively. During lactation, the blood levels of calcium, phosphorus, potassium and sodium in the same cows were 9.9, 4.35, 20.6 and 340 mg./ 100 ml., respectively (Kendall and Harshbarger, 1963). This decrease in serum calcium and phosphorus at parturition was found in nearly all cases of paresis. One early theory was that hypocalcaemia was caused by the large drain of body calcium to supply sufficient calcium for the colostrum milk. Moodie (1960) stated that in a number of cases which occur before calving the loss of calcium in the udder would not appear to exceed a few grams. However, Moodie (1960) stated that a cow secreting three gallons of colostrum milk/day would lose about 21 gm. of calcium/day through the udder. On the other hand, Ward et al. (1952) observed cows with a severe negative calcium balance prior to parturition.

Boda and Cole (1954) suggested that parturient paresis is associated with a failure of the parathyroid glands to function properly at the time of calving. The parathyroid glands secrete parathormone which is described as a coarse regulator of the blood calcium and calcitonin which is described as a fine regulator of the calcium homeostasis (Care et al., 1967). Parathyroidectomy of lactating cows resulted in a decrease in serum calcium for a few days and then the serum calcium came back up to normal (Stott and Smith, 1957[a]). The cows were rechecked to see if any of the glands were still present, but all tests proved negative. Mayer et al. (1967) observed an increase in fecal calcium when parathyroid extract was given to both intact and parathyroidectomized cows. In the parathyroidectomized cows the increased fecal calcium was a result of both decreased absorption and increased endogenous secretion. Also, in the parathyroidectomized cows it was observed that after the injections of parathyroid extract were stopped the plasma calcium decreased for about six days but then came back up to the same level as before the parathyroid gland was removed. No explanation was given as to why the cows returned to normal after the parathyroidectomization. The parathyroid glands appear to be essential for normal homeostasis in dairy calves (Stott and Smith, 1957[b]), but there remains some question as to the theory that parturient paresis is a result of a failure of the parathyroid glands in a mature cow.

Various studies have been conducted with different levels and ratios of calcium and phosphorus fed to dairy cows. Stott (1965) concluded that the lack of available dietary phosphorus during lactation is a predisposing factor in the cause of milk fever. The herd of Jerseys which had an incidence of 74% of milk fever were fed approximately

189 gm. of calcium and 51 gm. of phosphorus/day. This made a ratio of 3.7:1. The treatment ration consisted of a daily intake of 137 gm. of calcium and 85 gm. of phosphorus/day which resulted in a ratio of 1.6:1. With the ratio of 1.6:1 the incidence of milk fever dropped from 74% down to none in about 6 mo. After removal from the treatment ration the same cows had a 56% incidence of milk fever the following year.

Dry cows are usually fed a large amount of roughage such as alfalfa hay with a very wide ratio of calcium:phosphorus and a relatively low level of phosphorus. Kendall et al. (1965) found that when nine paretic suspects were fed grain for 3 weeks prepartum at a level of 1% of their body weight none of the suspects developed milk fever at parturition, whereas in the previous year the same cows which were fed a level of 0.5% grain had developed milk fever. In comparison, the serum calcium and phosphorus levels for the 1% group were 7.49 and 2.97 mg./100 ml., respectively, and the levels of 0.5% group were 5.42 and 1.20 mg./100 ml., respectively. Kendall et al. (1968) reported that three groups of cows consisting of 18, 15, and 15 cows in each group were fed three weeks prepartum a basal ration of alfalfa hay plus a grain mixture at the daily rate of 1% of body weight. The rations fed provided dietary ratios of calcium:phosphorus of 4.3:1, 0.91:1, and 2.3:1. The number of clinical parturient paresis cases in the respective groups were 8, 7 and 1. Boda and Cole (1954) found that low calcium and high phosphorus diets effectively prevented milk fever. The animals which received 118 gm. of calcium and 18 gm. of phosphorus/day had a 30% incidence of milk fever. However, the animals which received 6.1 gm. of calcium and 18.2 gm. of phosphorus/day had no incidence of milk fever. In the animals with no milk fever, there was hypertrophy of the parathyroid glands. As previously discussed, Wasserman (1962) stated that the presence of vitamin D in feedstuffs increases the amount of calcium absorbed. Hibbs and Conrad (1960) fed massive doses of vitamin D, i.e., 15, 20 and 30 million I.U./day, to cows which were suspects. Therefore, the larger doses protected about 50% of the herd against milk fever. It was noted that little protection was obtained before three days of feeding were completed and the protection was rapidly lost following cessation of vitamin D feeding. No adverse effects were noted after feeding that could be attributed to the large doses of vitamin D. Payne and Manston (1967) found that prolonged feeding of 20 million I.U. or more caused metastatic calcification and produced some lesions.

Later, Hibbs and Conrad (1966) found that grain feeding prepartum improved calcium and phosphorus metabolism. When the cows had a positive phosphorus balance, the feeding of 32,000 I.U. of vitamin D/lb. of grain increased calcium absorption but when the cows had a negative phosphorus balance, the feeding of vitamin D did not increase absorption. The feeding of vitamin D prevented milk fever in cows with a previous history, but did not prevent milk fever in cows which had no previous history of the disease.

CHAPTER III

EXPERIMENTAL PROCEDURE

Experimental Units

This report covers the first part of a long term calcium and phosphorus study with lactating dairy cows. Forty-eight cows (27 Holsteins, 15 Ayrshires and 6 Guernseys) were divided into blocks of three on the basis of breed, season of calving, lactation number and initial milk production. A randomized block design was used with the respective members of each trio assigned to ration treatments as indicated in Table I.

TABLE I

PLAN OF EXPERIMENT AND CALCULATED RATION ANALYSIS

Group	No. of cows	Ca:P ratio	Level in milking ration[a] Ca	P	Level in dry cow ration[b] Ca	P
			(%)	(%)	(%)	(%)
1	16	3:1	1:11	.37	.77	.26
2	16	3:1	1.63	.55	1.43	.48
3	16	1.5:1	.82	.56	.72	.48

[a]Milking ration consisted of a 50:50 ratio of grain and hay (alfalfa) allotted in accordance with the 1965 NRC standard.

[b]Ration for dry cows had a 25:75 ratio of grain to hay (½ alfalfa and ½ prairie), fed to maximum consumption.

The cows were started on the experiment three weeks after the Saturday nearest to the day of calving. Of the cows used on the experiment, 36 were in the first lactation and 12 were in the second lactation.

Feeding Regimen

Grain and alfalfa hay (50:50 ratio) were fed to maximum consumption during the first four weeks on experiment. At that time, it was assumed the cows were at the point of maximum production. Feed allowances were then calculated on the basis of size, age, milk production and fat percentage according to the 1965 N.R.C. standard. Peak production was calculated from the average of the first three days of the 8th week of lactation. For calculating feed allowances the average of the fat tests for the 6th, 7th, and 8th weeks was used. The grain was calculated to contain 72% TDN and the hay was estimated to contain 56% TDN making an estimated 64% TDN for the total ration. The cows in the second lactation were given a maintenance allowance half way between the requirement for growth and for maintenance. The requirement for growth was used as the maintenance requirement for the first calf hiefers. The feed allowances were reduced in equal increments in the succeeding 4-week intervals to allow for maintenance and production of 9.1 and 6.8 kg. of milk by the Holsteins and smaller breeds, respectively, during the tenth month of lactation.

After the tenth 4-week period of the experiment the cows were assigned to a dry cow ration. Grain and hay (25:75 ratio) were fed to maximum consumption during this period. Of the daily allotment of hay fed, one-half was alfalfa and the other half was prairie hay. The concentrate mixtures were adjusted as to maintain nearly the same calcium and phosphorus percentages as fed during the lactation period. After calving, the cows were again placed on the same rations which were fed during the first 40-week period of the experiment. Allowances were readjusted for size, age and production.

Concentrate Preparation

All grain rations for the experiment were mixed by Stillwater Milling Company, Stillwater, Oklahoma. Only one mixer was used and only one ration was prepared at a time. The mixer was always cleaned out with 150 lb. of crimped milo before the preparation of each ration. Mixing of the rations was supervised by a member of the Dairy Department. The mixing time was 5 minutes. Each sack of each ration was identified by a tag marked with a designated color.

Monosodium phosphate, calcium carbonate, and mono, dicalcium phosphate were used to supplement the rations to provide the desired amounts of calcium and phosphorus (Table II and III). The large amount of phosphorus provided by monosodium phosphate was used to balance the large amount of calcium found in alfalfa to obtain a narrow ratio of the two elements.

Management of the Cows

The cows were milked twice daily, at 2:00 A.M. and 2:00 P.M. The cows were kept in the two outside lots during the day. At 4:00 P.M. they were placed in individual tie stalls in a loafing barn adjacent to the lots to eat their daily allotment of hay. At the end of the four hours they were turned out into the lot for observation. Any cows that did not consume the entire allotment of hay in the afternoon were returned to the stalls in the morning for an opportunity to consume the

TABLE II

EXPERIMENTAL RATIONS FED DURING LACTATION (50:50 GRAIN TO HAY RATIO)

Ingredients	Ca:P ratio 3:1, low phosphorus			Ca:P ratio 3:1, high phosphorus			Ca:P ratio 1.5:1, high phosphorus		
	Feed	Ca	P	Feed	Ca	P	Feed	Ca	P
	kg.								
Alfalfa hay	1000	15.50	2.40	1000	15.50	2.40	1000	15.50	2.40
Sorghum grain	325	.09	.91	290	.08	.81	325	.09	.91
Barley	300	.18	1.20	300	.18	1.20	300	.18	1.20
Oats	100	.09	.33	100	.09	.33	100	.09	.33
Wheat bran	100	.13	1.29	100	.13	1.29	100	.13	1.29
Cottonseed meal	100	---	1.19	100	---	1.19	100	---	1.19
Molasses, liquid	50	.33	.04	50	.33	.04	50	.33	.04
Trace mineral salt	10	---	---	10	---	---	10	---	---
Mono, dicalcium phosphate	---	---	---	125	3.59	3.68	---	---	---
Calcium carbonate	15	5.85	---	325	12.68	---	---	---	---
Monosodium phosphate	---	---	---	---	---	---	15	---	3.83
Totals	2000	22.17	7.36	2000	32.58	10.94	2000	16.33	11.19
%		1.11	0.37		1.63	0.55		0.82	0.56

TABLE III

EXPERIMENTAL RATIONS FED AFTER 40 WEEKS (25:75 GRAIN TO HAY RATIO)

Ingredients	Ca:P ratio 3:1, low phosphorus			Ca:P ratio 3:1, high phosphorus			Ca:P ratio 1.5:1, high phosphorus		
	Feed	Ca	P	Feed	Ca	P	Feed	Ca	P
	kg								
Alfalfa hay	1500	23.25	3.60	1500	23.25	3.60	1500	23.25	3.60
Prairie hay	1500	4.95	1.80	1500	4.95	1.80	1500	4.95	1.80
Sorghum grain	320	.09	.87	248	.08	.70	305	.09	.86
Barley	300	.18	1.20	300	.18	1.20	300	.18	1.20
Oats	100	.09	.33	100	.09	.33	100	.09	.33
Wheat bran	100	.13	1.29	100	.13	1.29	100	.13	1.29
Cottonseed meal	100	---	1.19	100	---	1.19	100	---	1.19
Molasses, liquid	50	.33	.04	50	.33	.04	50	.33	.04
Trace mineral salt	10	---	---	10	---	---	10	---	---
Mono, dicalcium phosphate	12.5	2.56	2.63	42.5	8.71	8.93	---	---	---
Calcium carbonate	17.5	6.83	---	50	19.50	---	---	---	---
Monosodium phosphate	---	---	---	---	---	---	35	---	8.93
Totals	4000	38.41	12.95	4000	57.22	19.07	4000	29.02	19.23
%		.96	.32		1.43	.48		.73	.48

remainder of it. One-half of the grain allotment was fed one hour prior to each milking in conventional stanchions with the manger partitioned for individual feeding. Water was available in the stanchions where grain was fed and in the lots. Block salt was available to all cows in the lots. Orts of hay and grain were weighed once daily after the morning feeding. If more than 10% of either the hay or grain fed was left as a weighback, the total ration was reduced a reasonable amount until there was nearly complete consumption of the feed offered.

Observations for estrus and other conditions relating to reproduction were made at 8:00 A.M., 8:00 P.M. and during the day while working with the animals. The cows were bred at the first estrus following 50 days postpartum and at each subsequent estrus until conception. Artificial insemination was used for all services. All cows were examined by the veterinarian in charge of the O.S.U. herd prior to the time for the first breeding. Any cases of infection of the reproductive tract were treated as necessary. Other veterinary treatments of the cows were determined on the basis of reproductive performance as follows: cows bred, but diagnosed not pregnant at 50 days after breeding; cows not bred and not cycling for 50 days after calving, or cows that have gone for 50 days since the last cycle. Reproductive failures were defined as cows which failed to conceive to five services without treatment, and to an additional three services with prescribed treatment.

The cows were removed from the milking group if they produced less than 10 lb./day for three consecutive days. However, the designated milking cow rations were continued through 40 weeks whether the cows were milking or not. Treatment for mastitis was carried out as needed for the cows.

Data Collection

A hand grab sample was taken from each 100 lb. sack of grain as it was fed. The samples were stored in plastic bags and refrigerated. A composite sample was made for each two-week period of the experiment. The composite sample was ground in Wiley Mill with a 1 mm. sieve. Duplicate 1 gm. samples were dried for 10-12 hr. at 100°C for dry matter determination. The dried samples were ashed in a Hoskins Electric Muffle Oven for 4 hours at 600°C. The ash was dissolved in 1:3 HCl (1 part HCl to 3 parts distilled water) and transferred to a 50 ml. volumetric flask and made up to volume with distilled water. Total phosphorus was determined by the procedure outlined by Fiske and Subbarow (1925), using a Klett-Summerson colorimeter (Model No. 1019).

The calcium content was determined by using the procedure outlined by Willis (1960) using a Perkin Elmer 303 atomic absorption spectrophotometer.

Approximately one-half the bales of alfalfa and prairie hay fed were sampled at two-week intervals using a Penn. State hay sampler. After mixing, a hand grab sample was taken from the quantity of hay obtained from sampling. This sample was ground in a Wiley mill with a 1 mm. sieve.

Determinations of dry matter, phosphorus and calcium were made using the same procedure described above for grain, except that the ashed sample was made up to a volume of only 25 ml.

Milk production was weighed and recorded twice daily. Individual milk samples were obtained from four consecutive milkings beginning with Monday P.M. of each week. The four samples were composited in proportion to the milk weight corresponding with each sample. To determine total solids, 3 ml. of milk was placed in an aluminum foil dish and dried for 4 hr. at 100°C in a forced air oven. Milk fat was determined by the Babcock procedure.

Body weights were recorded for three consecutive days beginning at the time the animal was placed on experiment and thereafter at 4-week intervals. In the following lactation the cows were weighed at the time of calving, three weeks after the nearest Saturday to calving and four-week intervals thereafter.

Blood samples were taken at the end of either the first or second week on experiment and at 4-week intervals thereafter. For the second lactation, a blood sample was taken within 12 hr. after calving, either three or four weeks after the nearest Saturday to calving and at four-week intervals thereafter. Blood was collected from the jugular vein and allowed to coagulate. The blood clot was broken loose from the wall of the tube with a small needle and then centrifuged for 12 minutes. Serum phosphorus was determined as described by the procedure in Fiske and Subbarow (1925). Serum calcium, magnesium, potassium, copper, and zinc were determined with a Perkin Elmer 303 atomic absorption spectrophotometer.

CHAPTER IV

RESULTS AND DISCUSSION

Feed Consumption

The cows were fed a 50-50 hay to grain ratio during the 40-week period. The hay and grain were fed so there would be nearly maximum consumption of the amount offered each day. The small amount of hay refused usually consisted of coarse stems or pieces of moldy material.

When the cows did not consume the calculated allotment, the amount of hay and grain was reduced to the point of complete consumption. Some of the cows which received 10 kg. or more of hay per day would not consume the entire allotment in one feeding. They usually consumed the remainder of the hay in the morning.

The allotted amount of grain was consumed more readily than was the hay by the cows in each of the three treatment groups (Table IV).

TABLE IV

AVERAGE DAILY FEED INTAKE FOR WEEKS 1-40

Item	Treatment group[a]		
	1	2	3
Grain dry matter intake, kg.	6.8	6.3	6.8
Hay dry matter intake, kg.	6.7	6.1	6.6
Calcium intake, gm.	151	190	121
Phosphorus intake, gm.	55	75	85
Calcium:phosphorus ratio	2.7:1	2.5:1	1.4:1

[a]Group 1, Ca:P ratio 3:1, low phosphorus; Group 2, Ca:P ratio 3:1, high phosphorus; Group 3, Ca:P ratio 1.5:1, high phosphorus.

Thus, the percentage of concentrate consumed by Groups 1, 2 and 3 was 50.4, 50.8, and 50.7, respectively. The small deviation from the intended 50% of grain in the ration was at least partly responsible for the fact that the ratios of calcium:phosphorus actually consumed were slightly different from the intended ratios. The calculated calcium: phosphorus ratios for Groups 1, 2 and 3 were 3:1, 3:1 and 1.5:1; however, the actual intake of calcium and phosphorus were in ratios of 2.7:1, 2.5:1 and 1.4:1. Another factor accounting for the difference in calculated ratios and the ratios actually consumed in the case of Group 2 was a lower level of calcium in the concentrate ration. The calcium content of the hay was very close to the calculated values. The overall percentage of phosphorus in the rations for Groups 1, 2 and 3 was 0.407, 0.605 and 0.634, respectively. These levels were slightly higher than the calculated values (Table II), which also influenced the ratio of calcium to phosphorus consumed. As shown in Table XII in the Appendix, the intake was at a maximum near the peak of lactation and then slowly decreased. During the early parts of the lactation the cows generally did not consume the theoretical amount of feed needed, yet some weight gain was obtained by the end of 24 weeks (Table V). The feed allowances were calculated such that an allowance for growth was considered for all cows.

Milk Production

Lower milk production was observed in Group 2 than in the other groups (Tables V and VI). However, when the production was adjusted according to initial milk production, the differences in either actual milk production or solids corrected milk were not statistically

TABLE V

AVERAGE DAILY MILK PRODUCTION AND COMPOSITION ADJUSTED BY COVARIANCE ANALYSIS FOR INITIAL MILK PRODUCTION (WEEKS 1-24)

Group	Ca:P ratio	Initial milk production	Milk	Fat	Total solids	SCM[a]	Body weight change
		(kg)	(kg)	(%)	(%)	(kg)	(kg)
1	3:1	22.7	19.5	3.5	12.35	18.4	+28
2	3:1	20.6	17.3	3.6	12.61	16.5	+29
3	1.5:1	21.9	19.3	3.6	12.54	18.5	+36

[a]Solids Corrected Milk (kg) = 12.3 Fat (kg) + 6.56 Non-fat solids (kg) - 0.0752 Milk (kg). SCM formula from Tyrrell and Reid (1965).

TABLE VI

AVERAGE DAILY MILK PRODUCTION AND COMPOSITION (WEEKS 1-40)

Group	Ca:P ratio	Initial milk production	Milk	Fat	Total solids	SCM[a]	Body weight change
		(kg)	(kg)	(%)	(%)	(kg)	(kg)
1	3:1	22.7	17.0	3.7	12.52	16.2	+57
2	3:1	20.6	13.2	3.8	12.80	12.9	+68
3	1.5:1	21.9	16.2	3.7	12.66	15.7	+64

[a]Solids Corrected Milk (kg) = 12.3 Fat (kg) + 6.56 Non-fat solids (kg) - 0.0752 Milk (kg). SCM formula from Tyrrell and Reid (1965).

significant (Tables VII and VIII). There was no apparent difference among treatment groups in the per cent fat or in per cent total solids. The composition of the milk was typical of the respective breeds used (Table XIII in the Appendix), verifying that a 50:50 grain to hay ration is adequate and effective in terms of maintaining a normal milk fat test.

These results are in agreement with Converse (1954) and Fitch et al. (1932). Converse (1954) observed that when cows were fed rations containing only 0.26% calcium milk production was not impaired.

There were no apparent differences between the groups with respect to body weight changes. For the first 40 weeks of the experiment the cows gained approximately 60 kg. The weight gain could be due to increased body size and development of the fetus.

No cases of parturient paresis were observed in any of the 48 cows. At the time of calving the animals were starting either their second or third lactation. Stott (1965) indicated that the lack of available dietary phosphorus during lactation is a predisposing factor to the cause of milk fever, and Boda and Cole (1954) found that a low calcium and high phosphorus diet effectively prevented milk fever. It can be concluded that neither the level of calcium and phosphorus fed in the present study, nor the ratios fed were a predisposing factor to milk fever. However, it is possible that either a wider or more narrow ratio than was used in this study might be detrimental. Kendall et al. (1968) observed that when rations provided ratios of calcium:phosphorus of 4.3:1 or 0.91:1, a higher incidence of milk fever was observed than for the dietary ratio of 2.3:1.

Hansard et al. (1954) noted that as the animal gets older the true absorption of calcium decreases and the endogenous fecal calcium

TABLE VII

ANALYSIS OF COVARIANCE OF AVERAGE DAILY MILK PRODUCTION (WEEKS 1-24)

Source	d.f.	Sum of squares	Mean square	F[a]
Total	47	58,703		
Covariable[b]	1	48,675		
Blocks	15	8,087	539.1	1.83
Treatment	2	1,941	970.5	3.29
Error	29	8,552	294.5	

[a] $P_{2,29}.05 = 3.33$

[b] Data adjusted for initial milk production.

TABLE VIII

ANALYSIS OF COVARIANCE OF SOLIDS CORRECTED MILK (WEEKS 1-24)

Source	d.f.	Sum of squares	Mean square	F[a]
Total	47	34,760		
Covariable[b]	1	28,502		
Blocks	15	4,555	303.7	1.07
Treatment	2	1,702	851.1	3.01
Error	29	8,204	282.9	

[a] $P_{2,29}.05 = 3.33$

[b] Adjusted for initial milk production.

increases. The fact that none of the experimental animals were more than five years old at parturition, may partly account for the lack of any parturient paresis in the present experiment.

Blood Mineral Composition

There was no apparent difference in the blood serum calcium (Figure 1) in any of the three groups, but there was a highly significant difference ($P < .005$) in the inorganic blood serum phosphorus between the different levels of feed phosphorus (Table IX). It was apparent that the treatment effect was distinct after one week of the treatment ration. The inorganic blood phosphorus level can be associated with the dietary intake of phosphorus. The lower dietary intake of phosphorus by the group fed the ration with approximately 0.4% phosphorus resulted in a decreased serum phosphorus level.

TABLE IX

ANALYSIS OF VARIANCE FOR INORGANIC BLOOD SERUM PHOSPHORUS

Source	d.f.	Sum of squares	Mean square	F
Total	479	770.3		
Blocks (B)	15	148.6	9.91	2.52**
Treatment (T)	2	120.8	60.41	15.37***
B x T (error a)	30	117.9	3.93	
Periods (P)	9	53.1	5.90	2.52*
T x P	18	13.6	0.76	0.32
T x P x B + B x P (error b)	135	316.3	2.34	

*$P < .05$
**$P < .025$
***$P < .005$

These observations are in agreement with Hill (1961) and Duncan (1958), who concluded that plasma phosphorus reflects phosphorus intake

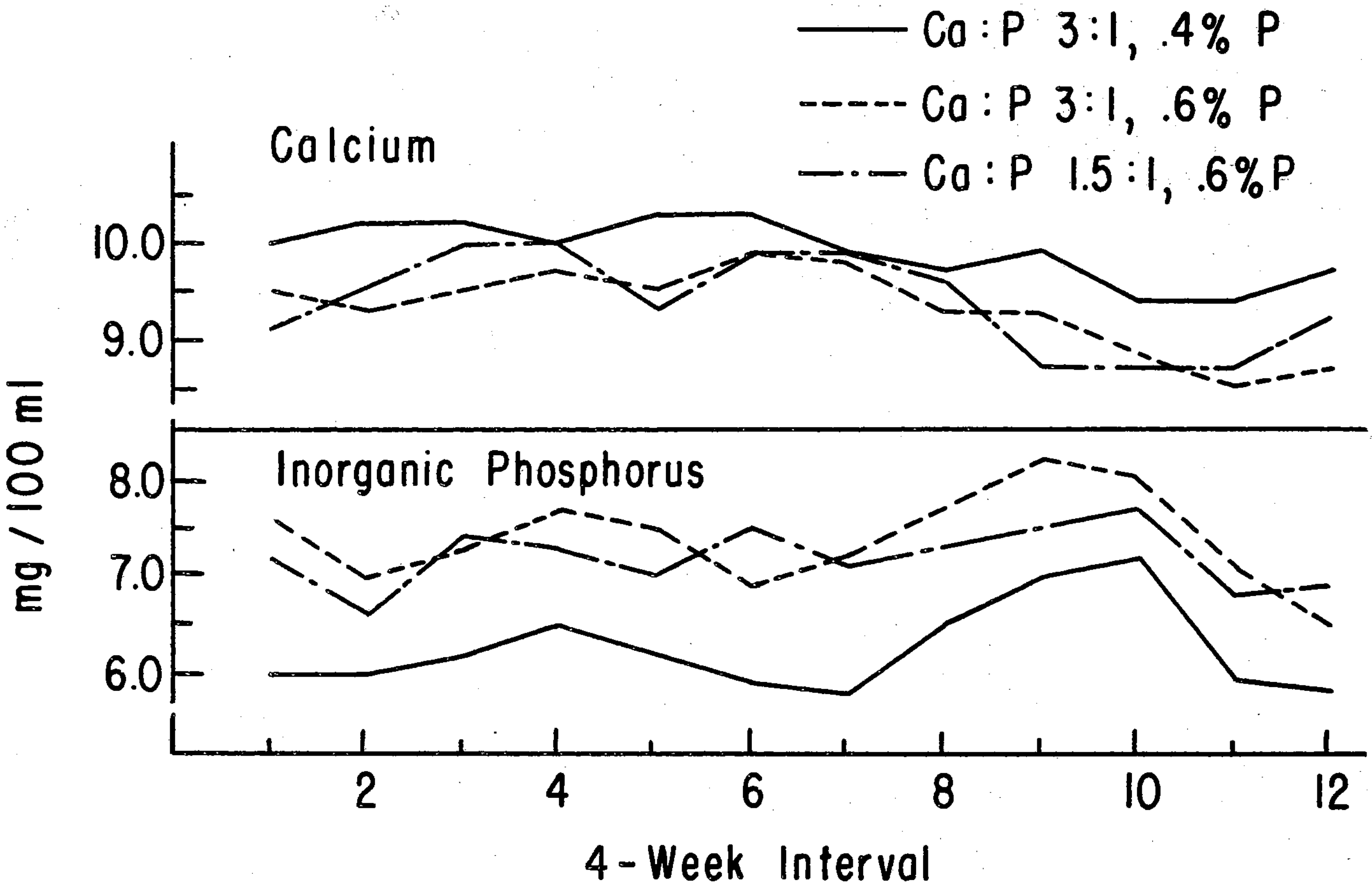

Figure 1. Concentration of Calcium and Inorganic Phosphorus in Blood Serum of Cows.

more rapidly than plasma calcium reflects calcium intake. A significant effect for periods was observed ($P < .05$). This is explained as a difference in blood phosphorus with respect to stage of lactation. Similar observations were made by Payne and Leech (1964) and Lane et al. (1968).

No apparent difference between treatments was observed for serum magnesium, potassium, zinc and copper (Figure 2). No explanation can be given for the drop in potassium during the later part of lactation.

Reproduction Performance

The number of services per conception for Groups 1, 2 and 3 were 2.56, 2.12 and 2.62, respectively. There was no apparent difference between any of the groups. The number of services per conception for individual cows varied from 1 to 8. As observed in Table XI in the Appendix, there were few cows in each group which required a high number of services per conception.

There was no appreciable difference between groups with respect to days from calving to first estrus, days from calving to first service and days from calving to conception (Table X). The number of days from calving to first estrus varied from 11 to 83 (Table XIV in the Appendix). Several of the cows that had a long interval to first estrus also required a larger number of services per conception. There appeared to be a notable amount of individual variation between the cows. The lack of any difference between the groups would be in agreement with other studies (Huffman et al., 1933; and Littlejohn and Lewis, 1960). Eckles et al. (1935) pointed out that reproductive disturbances under natural conditions are probably due to a large number of factors and not just a phosphorus deficiency alone. The levels of calcium and phosphorus fed

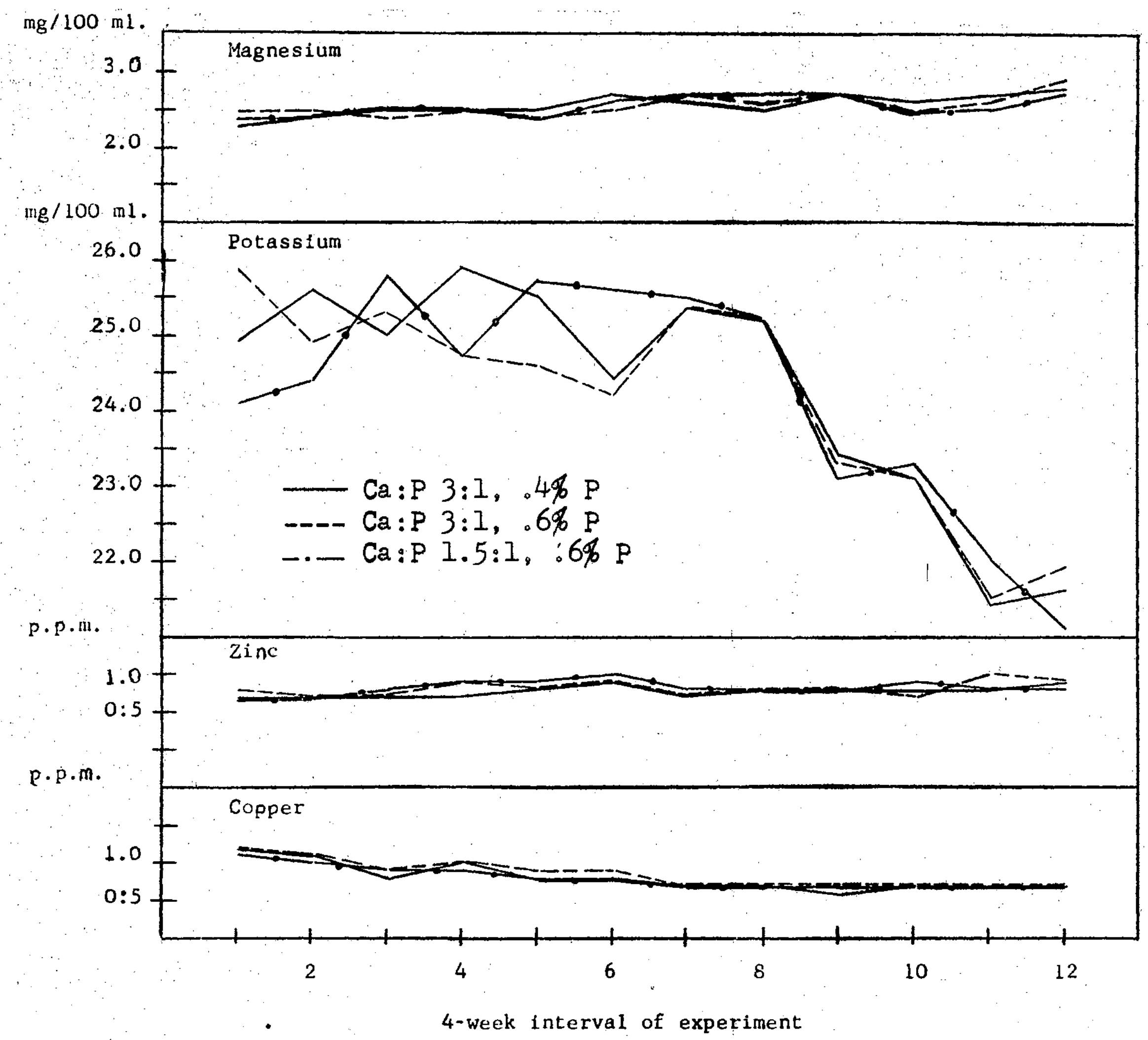

Figure 2. Concentration of various minerals in the Blood Serum of Cows.

were well above the minimum levels established by Palmer et al. (1935) and Fitch et al. (1932).

TABLE X

AVERAGE REPRODUCTIVE PERFORMANCE

Group[a]	Days from calving to first estrus	Days from calving to first service	Days from calving to conception	Services per conception
1	42.7	61.1	112.6	2.56
2	34.7	57.1	86.3	2.12
3	37.2	63.4	101.7	2.62

[a]Group 1, Ca:P ratio 3:1, low phosphorus; Group 2, Ca:P ratio 3:1, high phosphorus; Group 3, Ca:P ratio 1.5:1, high phosphorus.

A few cows were treated with antibiotics for infections of the reproductive tracts and a few of the repeat breeders were treated for cystic ovaries but there was no apparent indication of reproductive disturbances being restricted to any particular group (Table XIV in the Appendix).

CHAPTER V

SUMMARY AND CONCLUSIONS

This study was undertaken to examine the effect of different ratios and levels of calcium and phosphorus in relation to parturient paresis, reproduction, milk production and composition, and blood mineral composition in dairy cows.

Forty-eight cows (27 Holsteins, 15 Ayrshires and 6 Guernseys) were divided into blocks of three on the basis of breed, season of calving, lactation number and initial milk production. Of the cows used on the experiment, 36 were in the first lactation and 12 were in the second lactation. The respective members of each block were assigned to one of three rations consisting of (1) calcium:phosphorus ratio 3:1, 0.4% phosphorus; (2) 3:1 ratio, 0.6% phosphorus; and (3) 1.5:1 ratio, 0.6% phosphorus. This report covers the first 40 weeks of a long-term study.

There were no appreciable differences between groups with respect to milk yield and composition, or reproductive performance. No cases of parturient paresis were observed in any of the cows. There was a highly significant difference ($P < .005$) between treatment groups in inorganic blood serum phosphorus. No difference between treatment groups were observed for blood serum calcium, magnesium, potassium, copper and zinc.

LITERATURE CITED

Ammerman, C. B., R. M. Forbes, U. S. Garrigus, A. L. Newman, H. W. Norton and E. E. Hatfield. 1957. Ruminant utilization of inorganic phosphates. J. Animal Sci. 16:796.

Arrington, L. R., C. B. Ammerman, O. Yap, R. L. Shirley and G. D. Davis. 1962. Measurement of phosphorus availability for calves. J. Animal Sci. 21:987.

Becker, R. B., W. M. Neal and A. L. Shealy. 1934. Effect of calcium-deficient roughages upon milk yield and bone strength in cattle. J. Animal Sci. 17:1.

Boda, J. M. and H. H. Cole. 1954. The influence of dietary calcium and phosphorus on the incidence of milk fever in dairy cattle. J. Dairy Sci. 37:360.

Brown, W. H. and V. R. Smith. 1964. Response of normal and thyroparathyroidectomized dairy cows to infusion of disodium ethylenediamine tetraacetate and Ca 45. J. Dairy Sci. 47:613.

Care, A. D., T. Duncan and D. Webster. 1967. Thyrocalcitonin and its role in calcium homeostasis. J. Endocr. 37:155.

Comar, C. L., R. H. Wasserman and F. W. Lengemann. 1966. Effect of dietary calcium on secretion of strontium into milk. Health Physics, Pergamon Press. 12.

Converse, H. T. 1954. Calcium requirements of dairy cattle. U. S. D. A. Tech. Bul. No. 1092.

Dowe, T. W., J. Matsushima and V. H. Arthaud. 1957. The effects of excessive calcium when fed with adequate phosphorus in growing rations for beef calves. J. Animal Sci. 16:811.

Duckworth, J. and R. Hill. 1953. Storage of elements in the skeleton. Nutr. Abstr. and Rev. 23:1.

Duncan, Dorothy L. 1958. The interpretation of studies of calcium and phosphorus balance in ruminants. Nutr. Abstr. and Rev. 28:3.

Dutton, James E. and J. P. Fontenot. 1967. Effect of dietary organic phosphorus on magnesium metabolism in sheep. J. Animal Sci. 26:1409.

Eckles, C. H., L. S. Palmer, T. W. Gullickson, C. P. Fitch, W. L. Boyd, L. Bishop and J. W. Nelson. 1935. Effects of uncomplicated phosphorus deficiency on estrous cycle, reproduction, and composition of tissues of mature dairy cows. Cornell Vet. 25:22.

Eckles, C. H., R. B. Becker and L. S. Palmer. 1926. A mineral deficiency in the rations of cattle. Minn. Agr. Exp. Stat. Bul. 229.

Ellenberger, H. B., J. A. Newlander and C. H. Jones. 1950. Composition of the bodies of dairy cattle. Vermont Agr. Exp. Stat. Bul. 558.

Ellenberger, H. G., J. A. Newlander and C. H. Jones. 1931. Calcium and phosphorus requirements for dairy cows. Vermont Agri. Exp. Stat. Bul. 331.

Fiske, C. H. and Y. Subbarow. 1925. The colorimetric determination of phosphorus. J. Biol. Chem. 66:375.

Fitch, C. P., W. L. Boyd, C. H. Eckles, T. W. Gullickson and L. S. Palmer. 1932. Report of an experiment to determine the effect of a low calcium ration on reproduction in cattle. Cornell Vet. 22:156.

Forbes, E. B. and F. M. Beegle. 1916. The mineral metabolism of the milk cow. Ohio Agr. Exp. Stat. Bul. 295.

Hansard, S. L., C. L. Comar and M. P. Plumlee. 1954. The effects of age upon calcium utilization and maintenance requirements in the bovine. J. Animal Sci. 13:1.

Hansard, S. L., H. M. Crowder and W. A. Lyke. 1957. The biological availability of calcium in feeds for cattle. J. Animal Sci. 16:437.

Hart, E. B. 1932. The relation of nutrition to contagious cattle abortion. Wis. Res. Bul. 112.

Hibbs, J. W. and H. R. Conrad. 1960. Studies of milk fever in dairy cows. VI. Effect of three prepartal dosage levels of vitamin D on milk fever incidence. J. Dairy Sci. 43:1124.

Hibbs, J. W. and H. R. Conrad. 1966. II Calcium, phosphorus and vitamin D. J. of Dairy Sci. 49:243.

Hignett, S. L. 1959. Some nutritional and other interacting factors which may influence the fertility of cattle. Vet. Rec. 71:247.

Hignett, S. L. and P. G. Hignett. 1952. The influence of nutrition on reproductive efficiency in cattle. II. The effect the phosphorus intake on ovarian activity and fertility of heifers. Vet. Rec.

Hill, R. 1961. The provision and metabolism of calcium and phosphorus ruminants. World Rev., Nutr. and Dietetics. 3:129-148.

Huffman, C. F., C. S. Robinson and O. B. Winter. 1930. The calcium and phosphorus metabolism of heavily milking cows. J. Dairy Sci. 13:432.

Huffman, C. F., C. S. Robinson, C. W. Duncan, L. W. Lamb and M. F. Mason. 1933. Study of the phosphorus requirement of dairy cattle. I. Phosphorus requirement for growth and reproduction from three months of age to first calving. J. Dairy Sci. 16:203.

Kendall, K. A. and K. E. Harshbarger. 1963. Comparison of the mineral levels in parturient paretic cows at parturition and during lactation. J. Dairy Sci. 46:635.

Kendall, K. A., K. E. Harshbarger, R. L. Hays and E. E. Ormistom. 1965. Incidence of parturient paresis and blood composition of paretic suspects associated with prepartum level of concentrate feeding. J. Dairy Sci. 48:819.

Kendall, K. A., R. L. Hays and E. E. Ormiston. 1968. Postpartum serum calcium and phosphorus levels associated with calcium carbonate and monosodium phosphate feeding. J. Dairy Sci. 51:978.

Kleiber, M., A. H. Smith, N. P. Ralson and A. L. Block. 1951. Radiophosphorus P 32 as tracer for measuring endogenous phosphorus in cow's feces. J. Nutr. 45:253.

Lamb, L. W., O. B. Winter, C. W. Duncan, C. S. Robinson and C. F. Huffman. 1934. A study of the phosphorus requirement of dairy cattle. II. Phosphorus, calcium and nitrogen metabolism of dairy cattle when alfalfa furnishes the principle source of protein. J. Dairy Sci. 17:233.

Lane, A. G., J. R. Campbell and G. F. Krause. 1968. Blood mineral composition in ruminants. J. Animal Sci. 27:766.

Lengemann, F. W. 1965. Lack of effect of level of dietary calcium upon fecal endogenous calcium. J. Dairy Sci. 48:1718.

Littlejohn, A. I. and G. Lewis. 1960. Experimental studies of the relationship between the calcium-phosphorus ratio of the diet and fertility in heifers. Vet. Rec. 72:1137.

Long, T. A., A. D. Tillman, A. B. Nelson, Willis D. Gallup and Bill Davis. 1957. Availability of phosphorus in mineral supplements for beef cattle. J. Animal Sci. 16:444.

Lueker, C. E. and G. P. Lofgreen. 1961. Effects of intake and calcium to phosphorus ratio on absorption of these elements by sheep. J. Nutr. 74:233-238.

Malan, A. I. and H. H. Green. 1928. Studies in mineral metabolism. VII. The unknown phosphorus fraction of calf blood. J. Agr. Sci. 18:391.

Malan, A. I., H. H. Green and P. J. Du Toit. 1928. Studies in mineral metabolism. V. Composition of bovine blood on phosphorus deficient pasture. J. Agr. Sci. 18:376.

Manston, R. 1964. Investigation of the effects of vitamin D 3 on calcium and phosphorus metabolism in cows using calcium 45 and phosphorus 32. British Vet. J. 120:365.

Manston, R. 1967. The influence of dietary calcium and phosphorus concentration of their absorption in the cow. J. Agr. Sci. 68:263-268.

Mayer, G. P., C. F. Ramberg, Jr. and D. S. Kronfeld. 1967. Calcium metabolism and kinetics in intact and parothyroidectomized cows given parothyroid extract. J. Nutr. 92:253.

Meigs, E. B., W. A. Turner, T. S. Harding, A. M. Hartman and F. M. Grant. 1926. Calcium and phosphorus metabolism in dairy cows. J. Agr. Res. 32:833.

Meigs, E. B., W. A. Turner and E. A. Kane. 1935. The effects on calcium and phosphorus metabolism in dairy cows, of feeding low-calcium rations for long periods. J. Agr. Res. 51:1.

Moodie, E. W. 1960. Some aspects of hypocalcemia in cattle. Vet. Rec. 72:50.

Moodie, E. W. 1965. Modern trends in animal health and husbandry. Hypocalcemia and hypomagnesaemia. British Vet. J. 121:338.

National Research Council, U. S. 1965. Nutrient requirements of dairy cattle. Pub. No. 464.

Nelson, T. E. and A. D. Tillman. 1967. Calcium status on adult sheep. J. Animal Sci. 26:927.

Palmer, L. S., C. P. Fitch, T. W. Gullickson and W. L. Boyd. 1935. Supplementary report of an experiment to determine the effect of a low calcium ration on reproduction in cattle. Cornell Vet. 25:229.

Palmer, L. S., W. S. Cunningham and C. H. Eckles. 1930. Normal variations in the inorganic phosphorus of the blood of dairy cattle. J. Dairy Sci. 13:174.

Payne, J. M. and F. B. Leech. 1964. Factors affecting plasma calcium and inorganic phosphorus concentrations in the cow with particular reference to pregnancy, lactation and age. British Vet. J. 120:385.

Payne, J. M. and R. Manston. 1967. The safety of massive doses of vitamin D 3 in the prevention of milk fever. Vet. Rec. 81:214.

Pearse, A. G. E. 1966. Common cytochemical properties of cells producing polypeptide hormones, with particular reference to calcitonin and the thyroid C cells. Vet. Rec. 79:587.

Perry, S. C., R. G. Cragle, H. F. Downey, W. E. Stewart and C. E. Short. 1966. Effects of parathyroid extract on removal of Sr 89, Ca 45, and P 32 by hemodialysis from conscious calves. J. Dairy Sci. 49:674.

Pischke, L. D. and G. H. Stott. 1964. Relationship of the bovine parathyroids to calcium and phosphorus in the milk. J. Dairy Sci. 47:698.

Raun, A., E. Cheng and W. Burroughs. 1956. Phytate phosphorus hydrolysis and availability to rumen microorganisms. J. Agri. and Food Chem. 4:869.

Reid, J. T. 1962. Re-examination of the calcium requirements of dairy cows. Cornell Nutr. Conf., p. 144.

Stott, G. H. 1965. Parturient paresis related to dietary phosphorus. J. Dairy Sci. 48:1485.

Stott, G. H. and R. Smith. 1964. Histology, cytology, and size of the parathyroid in bovine related to age and function. J. Dairy Sci. 47:426.

Stott, G. H. and V. R. Smith. 1957[a]. Parturient paresis. results of parathyroidectomy of cows. J. Dairy Sci. 40:897.

Stott, G. H. and V. R. Smith. 1957[b]. Some results of parathyroidectomy of calves. J. Dairy Sci. 40:893.

Symonds, H. W., R. Manston, J. M. Payne and B. F. Sansom. 1966. Changes in the calcium and phosphorus requirements of the dairy cow at parturition with particular reference to the amounts supplied to the foetus in utero. Br. Vet. J. 122:196.

Tillman, A. D. and J. R. Brethour. 1958[a]. Dicalcium phosphate and phosphoric acid as phosphorus sources for beef cattle. J. Animal Sci. 17:100.

Tillman, A. D. and J. R. Brethour. 1958[b]. Utilization of phytin phosphorus by sheep. J. Animal Sci. 17:104.

Tillman, A. D., J. R. Brethour and S. L. Hansard. 1959. Comparative procedures for measuring the phosphorus requirement of cattle. J. Animal Sci. 18:249.

Tyrrell, H. F. and J. T. Reid. 1965. Prediction of the energy value of cow's milk. J. Dairy Sci. 48:1215.

Visek, W. K., R. A. Monroe, E. W. Swanson and C. L. Comar. 1953. Determination of endogenous fecal calcium in cattle by a simple isotope dilution method. J. Nutr. 50:23.

Ward, G. M., T. H. Blosser and M. F. Adams. 1952. The relation of prepartal and post-partal mineral balances to the occurrence of parturient paresis in dairy cows. J. Dairy Sci. 35:587.

Wasserman, R. H. 1962. Vitamin D 3 and the transfer of calcium across the intestinal membrane: A biophysical approach. Cornell Nutr. Conf.

Webster, Maurice W. 1932. Bovine sterility in New Zealand. Australian Vet. J. 8:199.

Westerlund, A. 1937. Numerical analysis of the Ohio investigations into the mineral metabolism of milch cows. Lantbrukshogskolans Ann. 4:55.

Westerlund A. 1956. The metabolic behavior of phosphorus. V. Kungl. Lantbrukshogskolans Ann. 22:317.

Willis, J. B. 1960. The determination of metals in blood serum by atomic absorption spectroscopy. I. Calcium Spectrochimica Acta. 16:259.

Wise, M. B., A. L. Ordoveza and E. R. Barrick. 1963. Influence of variations in dietary calcium:phosphorus ratio on performance and blood constituents of calves. J. Nutr. 79:79.

Wise, M. B., R. A. Wentworth and S. E. Smith. 1961. Availability of the phosphorus in various sources for calves. J. Animal Sci. 20:329.

A P P E N D I X

TABLE XI

NUMBER OF SERVICES PER CONCEPTION

Breed[b]	Block	Treatment Group[a] 1	2	3
H	1	7	2	3
H	2	1	1	1
H	3	1	1	3
H	9	3	1	1
H	11	5	2	1
H	12	1	1	5
H	13	2	1	1
H	15	4	2	4
H	16	1	1	1
	Mean	2.8	1.3	2.2
A	6	2	6	2
A	7	1	1	5
A	8	1	4	1
A	10	1	4	2
A	14	8	1	8
	Mean	2.6	3.2	3.6
G	4	2	2	2
G	5	1	4	2
	Mean	1.5	3.0	2.0

[a]Group 1, Ca:P ratio 3:1, low phosphorus; Group 2, Ca:P ratio 3:1, high phosphorus; Group 3, Ca:P ratio 1.5:1, high phosphorus.

[b]H = Holstein; A = Ayrshire; G = Guernsey.

TABLE XII

AVERAGE WEEKLY FEED CONSUMPTION

Weeks	Dry matter intake Grain	Hay	Ca intake	P intake	Ca:P ratio
Group 1[a]	kg		gm		
1	46.9	43.9	1054	358	2.9:1
2	50.3	47.7	1139	383	3.0:1
3	43.7	50.9	1219	405	3.0:1
4	55.2	53.1	1247	426	2.9:1
5	56.8	54.4	1275	442	2.9:1
6	55.5	52.3	1245	435	2.9:1
7	55.4	53.3	1250	439	2.8:1
8	56.8	54.2	1268	451	2.8:1
9	55.7	53.7	1262	437	2.9:1
10	55.6	53.8	1255	437	2.9:1
11	56.1	54.5	1255	448	2.8:1
12	56.2	54.3	1271	460	2.8:1
13	53.1	51.4	1216	443	2.7:1
14	53.5	51.8	1209	450	2.7:1
15	53.6	51.7	1200	458	2.6:1
16	53.4	51.7	1177	454	2.6:1
17	50.7	49.0	1110	427	2.6:1
18	50.8	49.7	1134	429	2.6:1
19	50.7	49.4	1144	424	2.7:1
20	50.6	49.2	1130	419	2.7:1
21	47.7	46.1	1024	392	2.6:1
22	47.9	46.3	1013	391	2.6:1
23	48.0	46.3	1013	393	2.6:1
24	47.4	45.8	1032	392	2.6:1
25	44.3	42.5	981	368	2.7:1
26	44.7	43.0	984	370	2.7:1
27	44.8	43.6	981	365	2.7:1
28	44.7	43.6	993	366	2.7:1
29	41.6	40.6	936	344	2.7:1
30	41.6	40.3	920	343	2.7:1
31	41.5	40.1	943	348	2.7:1
32	41.6	40.1	932	319	2.9:1
33	38.4	37.5	833	318	2.6:1
34	38.5	37.6	815	318	2.6:1
35	38.5	37.6	806	315	2.6:1
36	38.6	37.6	826	288	2.9:1
37	35.8	34.9	760	288	2.6:1
38	35.6	34.9	758	287	2.6:1
39	35.7	34.9	763	286	2.7:1
40	35.6	34.8	773	284	2.7:1

[a]Group 1, Ca:P ratio 3:1, low phosphorus; Group 2, Ca:P ratio 3:1, high phosphorus; Group 3, Ca:P ratio 1.5:1, high phosphorus.

TABLE XII (Continued)

Weeks	Dry matter intake Grain	Hay	Ca intake	P intake	Ca:P ratio
Group 2	—— kg ——		—— gm ——		
1	44.1	42.2	1412	510	2.8:1
2	47.7	44.8	1481	548	2.7:1
3	44.9	47.5	1535	587	2.6:1
4	52.4	51.0	1620	629	2.6:1
5	54.3	51.6	1665	642	2.6:1
6	52.4	49.5	1627	615	2.6:1
7	52.4	50.4	1609	608	2.6:1
8	51.4	50.6	1551	597	2.6:1
9	50.8	49.2	1519	584	2.6:1
10	50.8	49.3	1541	583	2.6:1
11	50.8	49.4	1560	604	2.6:1
12	50.7	49.1	1573	616	2.6:1
13	48.6	46.5	1509	598	2.5:1
14	48.9	47.1	1510	608	2.5:1
15	48.6	47.6	1495	606	2.5:1
16	48.0	47.6	1472	600	2.5:1
17	46.2	44.8	1404	579	2.4:1
18	45.8	44.4	1401	567	2.5:1
19	45.5	44.4	1392	557	2.5:1
20	45.7	44.9	1389	555	2.5:1
21	43.7	42.7	1314	534	2.5:1
22	43.7	42.5	1299	537	2.4:1
23	43.5	41.8	1263	514	2.5:1
24	43.6	41.8	1272	509	2.5:1
25	41.3	40.3	1219	476	2.6:1
26	41.3	40.3	1207	470	2.6:1
27	41.0	40.2	1211	473	2.6:1
28	40.6	39.8	1208	477	2.5:1
29	39.1	37.9	1158	461	2.5:1
30	38.9	37.8	1157	463	2.5:1
31	38.4	37.5	1146	462	2.5:1
32	38.7	37.2	1146	468	2.4:1
33	37.0	35.5	1069	440	2.4:1
34	36.9	35.8	1054	430	2.5:1
35	37.1	36.2	1078	441	2.4:1
36	36.7	36.1	1093	443	2.5:1
37	34.7	33.9	1038	417	2.5:1
38	34.5	33.8	1036	411	2.5:1
39	34.3	33.6	1046	403	2.6:1
40	33.4	33.1	1038	397	2.6:1

TABLE XII (Continued)

Weeks	Dry matter intake Grain	Hay	Ca intake	P intake	Ca:P ratio
Group 3	kg		gm		
1	45.4	43.4	827	528	1.6:1
2	49.9	47.6	898	585	1.5:1
3	52.7	49.7	937	616	1.5:1
4	54.8	51.9	990	637	1.6:1
5	55.3	53.1	1000	646	1.5:1
6	56.2	53.3	994	661	1.5:1
7	57.0	54.3	1031	683	1.5:1
8	57.4	55.6	1057	700	1.5:1
9	55.8	53.6	1020	679	1.5:1
10	55.8	54.3	1030	688	1.5:1
11	56.3	55.2	1020	705	1.4:1
12	56.5	55.6	1012	719	1.4:1
13	53.8	53.0	969	680	1.4:1
14	53.7	52.7	975	682	1.4:1
15	53.5	52.9	954	697	1.4:1
16	53.2	52.9	948	701	1.4:1
17	50.4	50.3	916	652	1.4:1
18	51.0	50.7	919	654	1.4:1
19	51.1	50.1	905	650	1.4:1
20	50.1	49.4	884	637	1.4:1
21	47.9	47.2	843	613	1.4:1
22	47.7	46.9	831	611	1.4:1
23	47.9	47.0	842	607	1.4:1
24	47.6	47.2	849	603	1.4:1
25	44.5	44.2	815	562	1.5:1
26	44.3	43.8	812	556	1.5:1
27	44.2	44.2	794	556	1.4:1
28	44.2	44.0	773	560	1.4:1
29	41.1	40.6	705	526	1.3:1
30	41.2	40.8	706	527	1.3:1
31	41.4	40.7	704	529	1.3:1
32	41.4	40.4	694	528	1.3:1
33	38.5	37.9	651	496	1.3:1
34	38.6	38.3	657	502	1.3:1
35	38.6	38.2	663	493	1.3:1
36	38.5	38.3	665	487	1.4:1
37	35.4	35.3	614	458	1.3:1
38	35.4	35.3	616	458	1.3:1
39	35.4	35.4	634	452	1.4:1
40	35.4	35.3	645	445	1.4:1

TABLE XIII

AVERAGE DAILY MILK PRODUCTION (WEEKS 1-40)

Cow No.	Milk	SCM[a]	Fat	Total Solids
Group 1[b]	kg	kg	%	%
684	17.7	16.1	3.4	11.99
669	19.0	15.3	3.6	12.52
685	20.5	17.8	3.2	11.62
704	17.6	18.2	4.2	13.21
756	10.1	12.0	4.9	14.91
691	15.8	14.9	3.5	12.48
692	14.6	14.0	3.6	12.59
623	15.9	11.6	2.8	9.41
668	14.1	13.5	3.6	12.59
658	12.6	12.9	4.1	13.28
627	18.7	17.9	3.6	12.51
611	19.8	19.5	3.8	12.79
710	21.3	19.5	3.3	12.17
755	15.6	15.2	3.7	12.80
718	24.9	21.8	3.2	11.64
610	18.0	16.2	3.4	11.87
Group 2				
663	15.9	15.4	3.6	12.61
662	14.5	14.8	3.9	13.27
693	13.6	12.6	3.2	12.38
681	12.2	14.5	5.0	14.91
688	11.3	12.9	4.8	14.39
739	9.4	10.0	4.4	13.48
679	13.6	13.4	3.8	12.90
617	6.7	6.3	3.6	12.42
702	12.3	11.7	3.5	12.73
689	14.4	13.9	3.7	12.55
607	13.5	12.9	3.6	12.57
646	4.9	4.6	3.4	12.35
741	21.6	19.5	3.2	12.05
705	13.5	13.8	4.1	13.07
724	15.7	15.2	3.8	12.49
632	17.9	15.7	3.2	11.72

[a]SCM = Solids corrected milk.

[b]Group 1, Ca:P ratio 3:1, low phosphorus; Group 2, Ca:P ratio 3:1, high phosphorus; Group 3, Ca:P ratio 1.5:1, high phosphorus.

TABLE XIII (Continued)

Cow No.	Milk	SCM	Fat	Total solids
Group 3	kg		%	
666	22.0	19.7	3.2	11.98
667	20.2	18.5	3.2	12.37
664	19.5	17.9	3.4	12.10
716	14.0	15.3	4.5	13.84
672	12.9	14.4	4.5	14.27
678	11.4	11.8	4.1	13.47
660	14.2	14.6	4.0	13.34
648	10.4	10.6	3.9	13.14
674	15.7	16.1	4.0	13.26
675	8.2	9.3	4.7	14.32
640	17.9	17.2	3.7	12.56
608	22.2	20.9	3.6	12.34
712	19.7	16.3	2.9	11.20
711	13.0	14.2	4.2	13.40
714	19.8	17.5	3.2	11.81
654	17.8	16.8	3.8	12.22

TABLE XIV

REPRODUCTIVE PERFORMANCE OF INDIVIDUAL COWS

Breed	Cow[a]	Date calved	Dates of pre-breeding cycles	Dates of cycles 50 days or more post-calving	No. Services	Remarks
H	684	6-27-66		9-18, 10-12,[b] 11-28, 12-31, 1-4, 2-7, 4-20, 5-29	7	ECP, 11-23; LH, 4-20
H	669	7-6-66	8-17	9-7, 10-30, 11-18	1	Aborted 11-6
H	685	7-16-66	8-24	9-12	1	
G	704	7-3-66	8-12, 8-19	9-2, 9-24	2	
G	756	7-7-66	8-20	9-9	1	
A	691	7-6-66		9-9, 10-2	2	
A	692	7-9-66	8-22	9-3	1	
A	623	8-1-66	9-4	9-27	1	
H	668	8-10-66	9-2, 9-10	10-2, 10-24, 11-13	3	
A	658	8-30-66	9-20, 10-5	10-28	1	
H	627	8-22-66	9-2, 9-18	10-13, 11-7, 11-11,[b] 11-20, 12-5, 1-24	5	LH 2-20 and 3-1
H	611	8-25-66	10-13	10-17	1	
H	710	9-22-66	11-5	12-5, 12-30	2	
A	755	10-21-66	11-24	12-18, 1-9, 2-1, 2-24, 3-20, 4-12, 5-5, 5-29	8	Hormone treatment 5-5 and 5-29
H	718	11-16-66		1-25, 2-9, 2-23,[b] 3-14, 5-28	4	LH, 1-19; penicillin 3-1, LH, 5-10
H	610	11-6-66	12-15	1-5	1	

[a]Group 1, Ca:P ratio 3:1, low phosphorus.

[b]In heat, but due to mismanagement was not bred.

TABLE XIV (Continued)

Breed	Cow[c]	Date calved	Dates of pre-breeding cycles	Dates of cycles 50 days or more post-calving	No. Services	Remarks
H	663	6-27-66		8-28, 9-24	2	
H	662	7-8-66	8-13	9-4	1	
H	693	7-23-66	8-15, 9-10	9-29	1	
G	681	7-2-66		8-21, 9-11	2	
G	688	7-19-66	8-14, 8-21	9-3, 9-23, 10-15, 11-10	4	
A	739	7-5-66		8-30, 9-23, 10-12, 11-2, 12-15, 1-28	6	Hormone treatment, 11-11
A	679	7-9-66		8-29	1	
A	617	7-28-66	8-25, 9-13	10-2, 10-22, 11-11, 11-30	4	
H	702	8-6-66	9-2, 9-7	9-26	1	
A	689	8-30-66	9-21, 10-7	10-29, 11-18, 12-15, 1-13	4	
H	607	8-28-66	9-29	10-21, 11-14	2	
H	646	9-3-66	9-29	10-23	1	
H	741	11-14-66	12-21	2-9	1	
A	705	11-11-66	12-15	1-3	1	
H	724	11-18-66	12-4	1-10, 2-19	2	Antibiotic treatment, 1-2
H	632	12-25-66	12-24	1-13	1	

[c]Group 2, Ca:P ratio 3:1, high phosphorus

TABLE XIV (Continued)

Breed	Cow[d]	Date calved	Dates of pre-breeding cycles	Dates of cycles 50 days or more post-calving	No. Services	Remarks
H	666	7-4-66	7-28, 8-18	9-11, 9-29, 10-20	3	ECP, 11-28 (by mistake)
H	667	7-11-66	8-16	9-8	1	
H	664	7-31-66	8-22, 9-2	9-23, 10-15, 11-6	3	
G	716	7-4-66	8-13	9-5, 9-27	2	
G	672	7-16-66		9-11, 10-17	2	
A	678	7-8-66	7-28, 8-16	9-5[b], 9-26, 10-17	2	
A	660	7-19-66	8-29	9-18, 11-11, 12-3, 12-23, 1-11	5	
A	648	9-13-66	10-2, 10-21	11-10	1	
H	674	8-16-66	9-24	10-16	1	
A	675	8-31-66	10-12	10-31, 11-19	2	
H	640	8-19-66	9-22	10-20[b], 11-27	1	
H	608	9-15-66		11-9, 12-5, 1-29[b], 3-2, 3-31, 4-23	5	
H	712	11-13-66	12-12	1-2	1	
A	711	12-6-66		1-24, 2-13, 2-24, 3-17, 4-8, 5-2, 5-24, 6-16	8	Hormone treatment, 5-2 and 5-24
H	714	12-9-66	1-21	2-14, 3-6, 3-28, 4-18	4	
H	654	12-12-66	1-25	2-19	1	

[d]Group 3, Ca:P ratio 1.5:1, high phosphorus

[b]In heat, but due to mismanagement was not bred.

VITA

Barry Jerome Steevens

Candidate for the Degree of

Master of Science

Thesis: CALCIUM AND PHOSPHORUS STUDIES WITH LACTATING DAIRY COWS

Major Field: Dairy Science

Biographical:

Personal Data: Born in Owen, Wisconsin, April 25, 1944.

Education: Graduated from Medford High School in 1962; received the Bachelor of Science degree from Wisconsin State University, River Falls, in 1966 with a major in Animal Science; completed requirements for the Master of Science degree from Oklahoma State University in July, 1968.

Experience: Raised on a dairy farm in Central Wisconsin, dairy and agronomy projects in 4-H and FFA from 1952-1962; worked in a cheese factory in 1961; Graduate Assistant in Dairy Science at Oklahoma State University in 1967 and 1968.

Member: Dairy Science Club, Zion Lutheran Church, Alpha Gamma Rho.